THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA.

VII. The Eyes of Amphisbæna punctata (Bell), A Blind Lizard From Cuba.[1]

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Amphisbæna punctata is a blind legless lizard which burrows in the ground. It is common in Cuba to which it is restricted. How deep it burrows, I do not know, but it is often turned out by the plow. The specimens obtained ranged from 103 to 245 mm. in length. The head is short, hard and pointed, and the tip of the upper jaw projects slightly beyond the tip of the lower jaw. In shape, arrangement of the dermal plates, and in the color of the ventral surface of the body it closely resembles an earthworm. The dorsal surface is flesh-color with small brown spots. The tail is short and flattened dorsoventrally. In a specimen 245 mm. in length, there were 225 annuli on the dorsal side, 202 on the ventral and 15 on the tail. In this specimen the tail was one thirteenth and the head one thirty-fifth the length of the body.

Methods. — The lizards were put alive into formalin. They were afterwards changed to alcohol. For decalcification, the heads were placed in five per cent. nitric acid from twenty to thirty days. A shorter period did not give satisfactory results. Some of the heads were imbedded in paraffin and others in paraffin and celloidin. In using the latter method I imbedded the head in celloidin in the usual manner and hardened in chloroform. From chloroform I transferred the block to soft paraffin for twenty-four hours and thence to hard paraffin for twenty-four hours, after which I imbedded the block in paraffin.

[1] Contribution from the Zoölogical laboratory of Indiana University, No. 77. The material used in the preparation of this paper was incidentally collected during several expeditions to Cuba. The prime object of the expeditions was to collect life history material of the Cuban blind fishes, *Lucifuga* and *Stygicola*. They were undertaken with a grant of $1,000 from the Carnegie Institution.

The best results were obtained from those imbedded in paraffin and celloidin. Several methods of staining were used; iron hæmatoxylin with eosin as a counterstain gave the best results. The more modern methods of treating the retina with silver could not be applied for lack of fresh specimens. On account of the extreme toughness of the cuticle it was impossible to get complete series of sections. For comparison I have examined the eye of *Anolis carolinensis*.

General Account of the Eye.

The eye of *Amphisbæna* appears indistinctly as a small black spot beneath the ocular plate (Fig. 1). In a specimen 225 mm. in length, the eye is 352 μ beneath the surface, 420 μ in width and 360 μ in depth. The conjunctival sac is 116 μ in diameter. The conjunctiva is very thin over the cornea, but measures 4 μ in thickness over the anterior part of the sac.

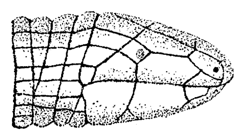

Fig. 1.

The dermis and epidermis have the same structure over the eye as over the regions near by. This corresponds with what Eigenmann ("The Eyes of *Rhineura floridana*," 1902) found in *Rhineura*, although the eye of *Rhineura* is a much more degenerate organ than the eye of *Amphisbæna*. To what extent the eye has degenerated from a more elaborate structure I am unable to say. Few organs are stationary and this one is probably still in process of reduction. I have been unable to obtain the young, and there is no means of finding out from the adult whether the eye is degenerating at present or not. In each specimen examined the eyes appeared in about the same state of degeneration.

The eye measures 1,224 μ in circumference and the pupil 104 μ in diameter. The uveal part of the iris on each side of the pupil measures 250 μ. The pupil and iris occupy 49.3 per cent. or very nearly half of the entire circumference.

Harder's gland is very much larger than the eye. In a cross-section through the central portion of the eye, the antero-posterior diameter is approximately three times and the medio-lateral diameter four times the medio-lateral diameter of the eye. It is

divided into two distinct lobes, the anterior being much smaller than the posterior. The gland completely surrounds the eye except over the anterior face. Its secretion is poured into the conjunctival sac and from thence into the mouth cavity. The large size of the gland in *Typhlops* led Duvernoy to the conclusion that its function was not connected with the eye. As its secretion, in *Amphisbæna*, is poured into the conjunctival sac and thence into the mouth cavity, its function must have been, primarily at least, connected with the eye.

No eye muscles are present in *Amphisbæna.*

The eye is directed outward and forward and makes an angle of about 60° with a line drawn tangent to the dermal plate which covers it.

Whether the eye is still used as a sense organ, I cannot say, but since the parts are so well developed and the eye is not buried very deeply beneath the surface, I am inclined to believe that it is at least, susceptible to light.

The Sclera. — The sclera (*scl.*, Fig. 6) has apparently undergone no degeneration whatever. It compares favorably with that of *Anolis.* In fact, there is but little difference in its structure in the two eyes. At the proximal part of the eye, the sclera measures 12 μ in thickness, while at the same place in *Anolis* it measures 15 μ. It is continuous over the front of the lens as the cornea, which together with the thin wall of the conjunctival sac at this place measures 7 μ. Scleral cartilages extend from about the middle of the eye back almost to the optic nerve. On each side of the sclera and forming a part of it, are thin irregular layers of pigment in patches.

The Choroid. — If the blood vessels in the choroid still persist, the preparations do not show them. All that can be seen is a number of densely pigmented cells, around and between which are filaments of connective tissue (*chr.*, Fig. 6). At the entrance of the optic nerve, this layer measures 8 μ in thickness, but gradually becomes less forward and vanishes entirely a short distance back of the enlarged end of the pigment layer. The pecten, present in *Anolis*, is not apparent in *Amphisbæna.*

The Lens. — The lens has retained its natural shape and position (*lens*, Fig. 6). It is almost spherical and measures 80 μ in

diameter. In most of the sections an outer layer of cells extends around the anterior surface of the lens. The interior in nearly every case stained as a structureless mass, but in a few sections it appeared to be made up of large irregularly shaped cells with small nuclei. If any fibrous cells still persist, they did not show. No capsule is present.

The Vitreous Body. — The vitreous body (*vit. cav.*, Fig. 6) occupies the greater part of the eye-ball and has certainly undergone but little change. The aqueous cavity has entirely disappeared.

The Iris. — Only the uveal part of the iris remains. It is continuous with the pigment epithelium of the retina and has the same structure. In the thickest part it measures 68 μ. The cells are similar to those of the pigment layer, except that their radial diameter is much greater. The ciliary processes are no longer present.

The Optic Nerve. — The optic nerve can be traced from the eye, through and along the side of Harder's gland. While the nerve could be traced no further on account of an incomplete series of sections, there is no doubt that the connection with the brain still exists. The nerve fibers enter the eye in a compact mass, pass through the layers of the retina until they reach the nerve fiber layer, where they spread out and connect with the nerve cells of the ganglionic layer in the usual manner.

The Retina.

While the retina has undergone considerable change, all the layers are still present (Fig. 3). It measures 78 μ in thickness. In *Anolis* about half way between the anterior and posterior parts of the eye it is 179 μ in thickness. If the macula lutea is still present, the preparations do not show it.

The Pigment Layer. — The pigment layer (1, Fig. 6) which bounds the retina externally, consists of a single stratum of rectangular cells separated by a small amount of clear intercellular substance. These cells have large oval nuclei free from pigment, almost transparent and with small nucleoli. At the back portion of the eye where the pigment layer measures 8 μ, the transverse diameter of the cells is greater than the radial diameter, but

toward the anterior portion where the layer becomes thicker, the radial diameter becomes much the greater. The greatest thickness of this layer is near the lens, where it measures 68 μ. The outer surface of the pigment cells — that which lies next to the choroid — is smooth and slightly convex. The inner surface, on the other hand, is very irregular. The cells at this place are very densely laden with pigment and prolonged into filamentous processes which extend between and amongst the cones. In fact, the cones may be said to be imbedded in the pigment cells. This layer differs but little from that of *Anolis*, except at the anterior part of the eye where it becomes much thicker.

The Cones. — No rods are present. The cones (2, Fig. 3) consist of an upper and a basal portion. The basal part is elliptical in shape and stains uniformly throughout, while the outer portion is longer and somewhat triangular in shape, with the smaller side of the triangle resting on the inner elliptical part. This layer measures 10 μ in depth while the same layer in *Anolis* measures 13 μ.

The Outer Nuclear Layer. — This layer is made up of a single stratum of nuclei with small dark nucleoli (3, Fig. 3). Some of these nuclei are almost spherical, while others are oval in shape. They are connected with the cones by broad processes which stain darkly. These processes may be very short, in which case the cone comes in close proximity to the nucleus; or they may be drawn out into filaments as long as or longer than the nuclei themselves. From the inner part of the nuclei extend processes which broaden toward the base and send numerous ramifications into the inner stratum of the outer reticular layer. There is a striking difference here between this eye and the normal one. The processes from the base of the nuclei pass straight through the outer reticular layer while in my sections of the normal eye, they pass through at an angle of about 45° (3, Fig. 4).

The Outer Reticular Layer. — The outer reticular layer (4, Fig. 3), is penetrated by the processes from the nuclei of the outer nuclear layer and by a few Müllerian fibers. If processes from horizontal cells are present they were not brought out by the method of staining which was used. Again, there is but little

difference in the thickness of this layer in the two eyes, as it measures 6 μ in *Amphisbæna* and 7 μ in *Anolis*.

The Inner Nuclear Layer. — The inner nuclear layer is a compact mass of somewhat irregular spherical nuclei and is 24 μ in thickness (6, Fig. 3). The corresponding layer in *Anolis* is 59 μ. Spongioblast and bipolar cells cannot be differentiated from each other. All the nuclei appear to be very much alike, except the nucleated enlargements of the fibers of Müller, which have no definite shape and which stain very densely. However, some nuclei, more especially those of the inner stratum, stain a very deep black color, and show no structure whatever. Parts of certain other nuclei stain densely, while the rest retains its original identity. Some of the nuclei have from four to six nucleoli. In *Anolis* two other kinds of nuclei appear. A few flattened horizontal nuclei can be seen near the middle of the layer and in the inner stratum are a number of large spherical nuclei. Penetrating this layer are many fibers of Müller. Each fiber as it passes through is characterized by a nucleated enlargement.

The Inner Reticular Layer. — The inner reticular layer measures 20 μ in thickness as against 45 μ in *Anolis* (8, Figs. 3 and 4). The method of staining brought out no definite structures. The fibers of Müller pass through it as fine vertical filaments. Occasionally there is a nucleus from the nuclear layer or from the ganglionic layer which lies imbedded in the edge of this layer.

The Ganglionic Layer. — The ganglionic layer (9, Fig. 3) consists of a single layer of nuclei 6 μ in diameter, with now and then another nucleus above or below the single layer. From the outer side of these nuclei fibers which run out and penetrate the inner reticular layer, can be traced for a short distance. On the opposite side are also fibers which continue as fibers of the nerve fiber layer. In *Anolis* this layer measures 23 μ and is made up of loosely connected nuclei, some of which are large and spherical, others are smaller and irregular, while still others stain very densely.

The Nerve Fiber Layer. — The nerve fiber layer is 6 μ in depth while in *Anolis* it is 26 μ.

The Fibers of Müller. — The Müllerian fibers can be traced from the membrana limitans interna to the outer nuclear layer.

They commence at the inner surface of the retina by a broad conical foot which extends into the ganglionic layer. Through the inner reticular layer the fibers pass as fine filaments, but in the inner nuclear layer each fiber is characterized by an irregularly shaped nucleus, which stains densely and shows no structure. The membrana limitans externa is not visible. These fibers differ but little from those in *Anolis*, except that those in *Anolis* can be traced to the membrana limitans externa, which is plainly visible.

Summary.

1. The eye muscles have entirely disappeared.

2. Only the uveal parts of the iris remain.

3. The lens has retained its shape and position, but its structure has been greatly changed. No capsule is present.

4. Harder's gland is many times larger than the eye and pours its secretion into the conjunctival cavity and thence into the mouth.

5. The sclera, scleral cartilages, cornea, vitrous body and pigment epithelium have undergone but little change unless it be in the reduction in size.

6. The cuticle passes over the eye unchanged.

7. The aqueous cavity is no longer present.

8. All the layers of the retina are still present. As shown in Fig. 6, the great reductions in the depth of the layers, in comparison with those of *Anolis*, has taken place in the nerve fiber, ganglion cell, inner reticular and inner nuclear layers.

9. If the eye has been reduced from an eye of the average size, all parts have certainly undergone considerable change, and this change has been approximately equal among the several parts.

10. The retina does not show such a profound change as either the iris, muscles or lens. However, it has been greatly changed, as it extends only 50.7 per cent. of the distance around the eye.

11. The eye of *Amphisbæna* bears out the statement made by Eigenmann ("Eyes of the Blind Vertebrates of North America," I.) that the more active parts of the eye are the ones to degenerate first. They are the parts which have been most affected.

Acknowledgments.

I took up the work on this lizard at the suggestion of Dr. C. H. Eigenmann and it is under his direction that the work has been carried forward. To him I am indebted for many helpful suggestions and for my specimens. I also wish to express my thanks to Mr. Leonard Haseman, of Lake City, Florida, for sending me specimens of *Anolis*.

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Explanation of Plate I.

1, pigment layer; 2, cones; 3, outer nuclear layer; 4, outer reticular layer; 6, inner nuclear layer; 8, inner reticular layer; 9, ganglion cell layer; 10, fiber layer; *lens*, lens; *scl.*, sclerotic; *chr.*, choroid; *cor.*, cornea; *scl.c.*, scleral cartilage; *n.op.*, optic nerve; *vit.cav.*, vitreous cavity; *con.cav.*, conjunctival cavity; *C.*, outer covering of the eye; *M.*, Müllerian fiber; *L.*, membrana limitans externa.

Figs. 1, 2 and 5 were drawn from sections. One-twelfth objective and one-inch eye-piece.

Figs. 4 and 6 are diagrammatic.

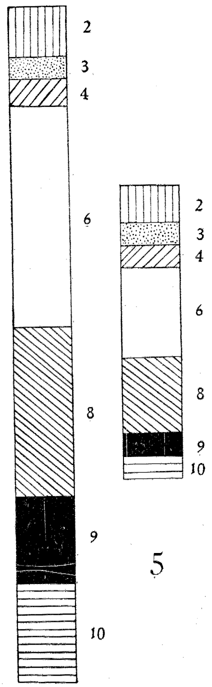
Fig. 1. Side view of the head.

Fig. 2. Diagram of the eye showing the parts in their relation and the distance of the eye beneath the surface.

Fig. 3. Horizontal section of the retina of *Amphisbæna punctata*, showing the different layers.

Fig. 4. Horizontal section of the retina of *Anolis*.

Fig. 5. Diagram showing the comparative measurements of the retina in the two eyes.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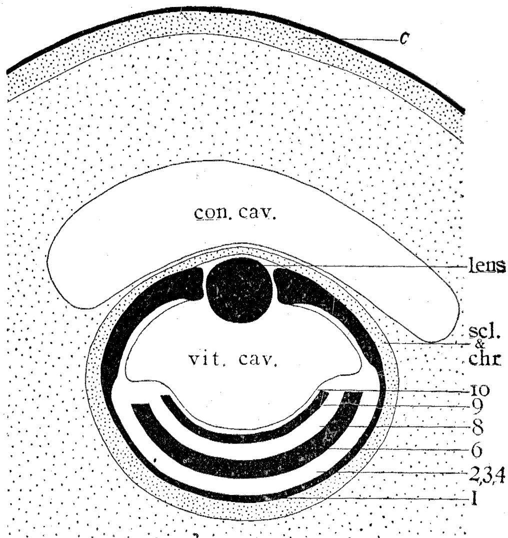
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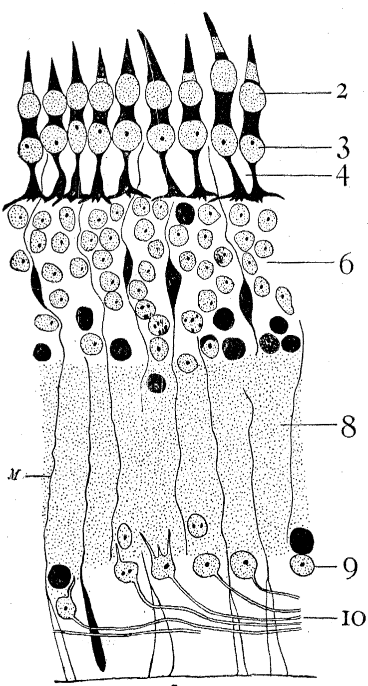
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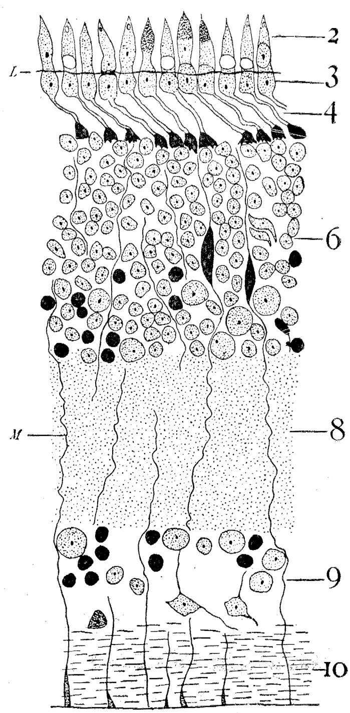
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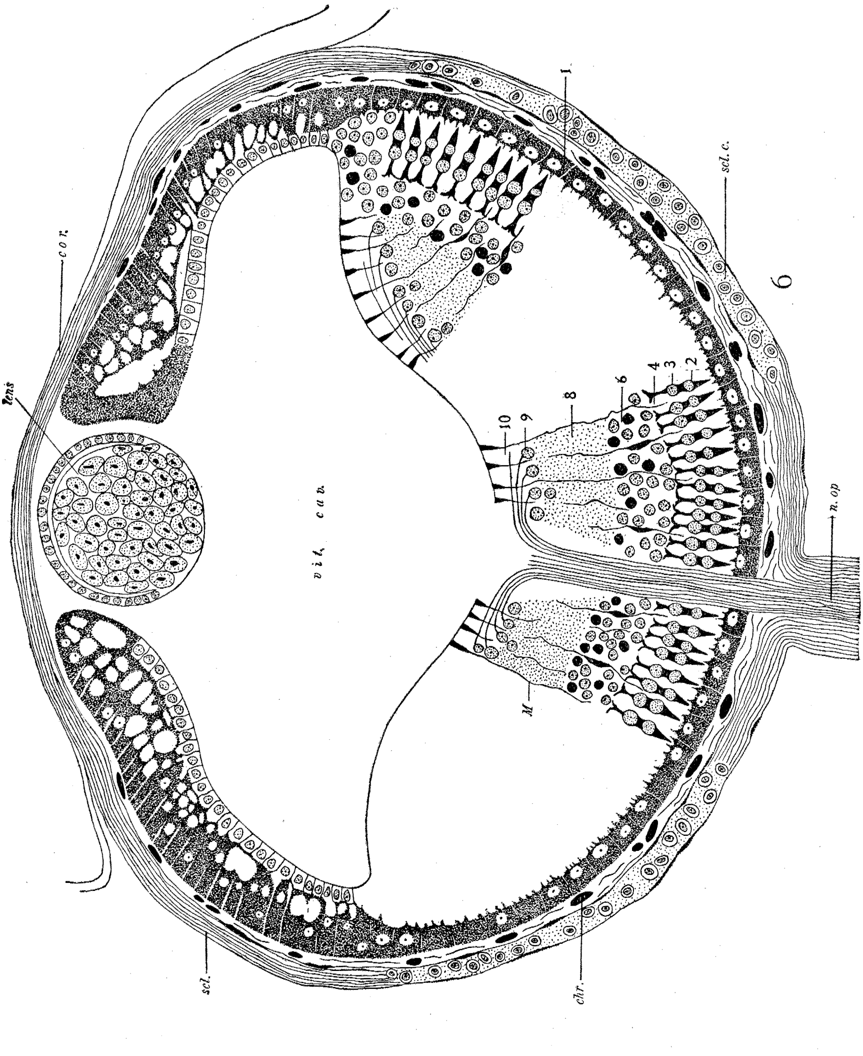
Explanation of Plate II.

Fig. 6. Horizontal section of the eye showing the different parts. The retina is diagrammatic.



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